Cancer Causes and Control **13**: 637–645, 2002.

Cigarette smoking, glutathione-*S*-transferase M1 and T1 genetic polymorphisms, and breast cancer risk (United States)

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Received 4 September 2001; accepted in revised form 3 April 2002

Key words: breast cancer, case–control, cigarette smoking, GSTM1, GSTT1.

Abstract

Objective: It has been suggested that functional polymorphisms in genes encoding tobacco carcinogen-metabolizing enzymes may modify the relationship between tobacco smoking and breast cancer risk. We sought to determine if there is a gene–environment interaction between GSTM1 (GSTM1A and GSTM1B), and GSTT1 genotypes and cigarette smoking in the risk of breast cancer.
Methods: Cases and controls were recruited in a case–control study conducted in Connecticut from 1994 to 1998. Cases were histologically confirmed, incident breast cancer patients, and controls were randomly selected from women histologically confirmed to be without breast cancer. A total of 338 cases and 345 controls were genotyped for GSTM1 and GSTT1.
Results: None of the GSTM1 genotypes, either alone or in combination with cigarette smoking, was associated with breast cancer risk. There was, however, a significantly increased risk of breast cancer among postmenopausal women with a GSTT1 null genotype (OR = 1.9, 95% CI 1.2–2.9). There were also indications of increased risk of breast cancer associated with cigarette smoking for postmenopausal women with GSTT1-null genotype, especially for those who commenced smoking before age 18 (OR = 2.9, 95% CI 1.0–8.8).
Conclusion: Women with a GSTT1-null genotype may have an increased breast cancer risk, especially postmenopausal women who started smoking at younger ages.

Introduction

It has been suggested that cigarette smoking may increase a women's risk of breast cancer [1]. This is biologically plausible since cigarette smoke contains many known or suspected human carcinogens [2], and the developing breast is particularly susceptible to cancer initiation [3]. Furthermore, a major class of carcinogens, namely polycyclic aromatic hydrocarbons (PAHs) abundant in cigarette smoke, have been shown to cause mammary tumors in rodents [4, 5] and to form DNA adducts in human breast cells [6, 7]. In addition, the PAH-induced DNA adduct levels in normal breast tissue were shown to be much higher in breast cancer cases than in corresponding controls [8]. Epidemiological studies of both active and environmental tobacco smoking and breast cancer, however, have to date produced inconsistent results, with some suggesting an increased risk, others no association, and a few showing a decreased risk, as recently reviewed by Johnson *et al.* [9] and Lash and Aschengrau [10].

In attempts to clarify the apparent discordance between these studies it has been suggested that functional polymorphisms in genes encoding tobacco carcinogen-metabolizing enzymes may modify the relationship between tobacco smoking and an individual's breast

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cancer risk [11, 12]. One such class of genes is those encoding the glutathione-*S*-transferases (GSTs). GSTs detoxify reactive chemical species, such as PAH epoxides, by catalyzing their conjugation with glutathione [13].

There are several members of the multigene GST family and all have been shown to be genetically polymorphic. Several GSTs, including GSTM1 and GSTT1, metabolize components of cigarette smoke [13]. For example, GSTM1 metabolizes and detoxifies benzo(*a*)pyrene-diol epoxides [14, 15], while GSTT1 is known to detoxify a wide range of potential carcinogens, including epoxides and constituents of tobacco smoke, such as alkyl halides [16, 17]. Therefore, individuals who lack GSTM1 and GSTT1 gene products, or express variants with a low metabolizing activity, may be at a particularly increased cancer risk after exposure to tobacco smoke [13, 18, 19].

Several recent reports have investigated the association between tobacco smoking, breast cancer, and GSTM1 and/or GSTT1 genetic polymorphisms [11, 20–26]. The results of these studies have been inconsistent, probably due to poor characterizations of exposure (such as classifying individuals as ''ever'' or ''never'' smokers). None of the studies has analyzed the data by GSTM1A and GSTM1B alleles.

Recent studies, however, suggest that analyses based on only GSTM1-positive or null genotype without any consideration of the GSTM1 allelotype may be too simplistic in defining the relationship between GSTM1 genotype and cancer risk, since the role of two alleles of the GSTM1-positive genotype (GSTM1A and GSTM1B) in detoxifying carcinogens is not well understood, leaving open the possibility that their effects may differ [27–30]. Therefore, in this study the GSTM1-positive genotype was further analyzed with respect to the GSTM1A and GSTM1B allelotypes.

We describe a case–control study that seeks to determine if the polymorphisms of GSTM1 and GSTT1 modify the relationship between cigarette smoking and breast cancer risk, based on major characteristics of cigarette smoking, such as age at start of smoking, duration of smoking, and amount of daily or lifetime smoking. These characteristics have been shown to be important in determining an individual's breast cancer risk from cigarette smoking [3, 31].

Materials and methods

Study subjects

This case–control study was designed to examine the potential role of GSTs on the risk of breast cancer and to test if the polymorphisms of GSTs modify the relationships between various environmental factors and breast cancer risk. The blood samples and information on environmental exposures were collected from a recently completed case–control study of female breast cancer in Connecticut, USA. The study population and methods used have been described in detail elsewhere [32, 33]. Briefly, cases were histologically confirmed, incident breast cancer patients who had a breast-related surgery at the Yale–New Haven Hospital (YNHH), in New Haven County, Connecticut in 1994–1997. Cases were 30–80 years old, had no previous diagnosis of cancer, with the exception of non-melanoma skin cancer, were alive at the time of interview, and were willing to donate blood for study purposes.

Potentially eligible cases were identified using computerized patient information from YNHH, where records of all newly completed breast-related surgeries are kept. We consecutively selected all breast cancer patients who met the study eligibility requirements as described above. A total of 338 incident breast cancer cases were recruited. The participation rate was 77% for cases.

Because of the high incidence rate of breast cancer and proliferative benign breast disease with atypia, we randomly selected controls from the same computerized files from women who had breast-related surgery but who were histologically confirmed to be without breast cancer. We used this strategy to select controls with the intention of avoiding selection of controls with non-diagnosed early-stage breast cancer or precancerous conditions, thereby reducing misclassification of disease status. A total of 345 controls were selected, frequency matched to the cases by age, within five-year intervals (30–34, ...). The participation rate was 71% for controls.

Interviews

After approval by each subject's hospital and physician, potential participants were approached by letter and then by phone. Those who consented were interviewed by a trained interviewer, either in-home or at a location convenient for the patient. A standardized, structured questionnaire was used to obtain information on tobacco smoking, alcohol drinking, menstrual and reproductive history, lactation history, past medical history, family cancer history, occupation, diet, and demographic factors. Information on cigarette smoking included age at start of smoking, amount of smoking per day, and the duration of smoking in years.

Blood collection and laboratory analysis of GST genotypes

Following the interview the participant provided a blood sample, collected by venipuncture by our study staff. Blood samples were held in a cooler until serum was separated, usually within 1–3 h. The blood clot samples were then coded and stored in our study freezer at −84 °C, until they were sent in batches to the study laboratory at the University of Texas, at M. D. Anderson Cancer Center.

To isolate high molecular weight genomic DNA for GST genotyping, the blood clots were thawed at room temperature, finely minced with sterile scalpels and re-suspended in 10 mM Tris-HCl (pH 7.4) containing 10 mM EDTA, 150 mM NaCl, 0.5% sodium dodecyl sulfate, and 1 mg/ml proteinase K. After incubation (37 °C; gentle shaking) for 1 h, the DNA was precipitated using the standard phenol–chloroform as previously described [34, 35]. The DNA was precipitated with one-tenth volume of 3 M sodium acetate and two volumes of 100% ethanol, washed with 70% ethanol, and re-dissolved in Tris-EDTA buffer (pH 7.2). DNA purity and yield were assessed by determining the optical densities at 260 nm and 280 nm.

Genotyping of GSTM1 and GSTT1 was performed using combinations of PCR and RFLP analysis, a modification of a previously described method [36]. Amplification was performed with the GSTM1 and GSTT1 specific primers. The PCR product was electrophoresed in 2% agarose, stained in 0.5% ethidium bromide, and photographed under UV illumination. Cell lines (human malignant gliomas and breast carcinoma) available in our study laboratory and representing GSTI-positive, GSTT1-null, GSTM1A, GSTM1B, and GSTM1-null polymorphisms were used as positive controls. Quality control procedures implemented for the GST genotype analyses included running of controls of the stable human cancer cell lines with known polymorphic GST gene and reanalyzing samples that yielded ambiguous result. Analyses of GST genes were performed in batches of 12 samples at a time. Samples were coded and batched at Yale, and the laboratory personnel at the M. D. Anderson Cancer Center, where the genotype analysis were done, were blinded to the sample identity.

Data analysis

Unconditional logistic regression was used to estimate the association between GSTM1 and GSTT1 genetic polymorphisms and breast cancer risk, to evaluate the modification of the effect of tobacco smoking on the risk of breast cancer by GST genotypes, and to control for potential confounders. A smoker was defined as someone who had smoked a total of 100 or more cigarettes during his/her lifetime. A current smoker was defined as someone who had smoked within the year preceding diagnosis, and an ex-smoker as someone who had smoked but who had quit more than one year prior to diagnosis. Overall, tobacco smoking was examined both in terms of the average number of cigarettes smoked per day and the pack-years smoked. The total pack-years was calculated as the average number of packs consumed per day multiplied by the number of years that amount was smoked: one pack-year is equivalent to 7300 cigarettes smoked.

Variables included in the final model were age (as a continuous variable), body mass index (<21, 21–24, ≥25 kg/m^2), lifetime months of lactation (0, 1–5, 6–11, ≥12 months), age at first full-term pregnancy (nulliparous, <20, 20–25, >25 years), family breast cancer history, and menopausal status. Other variables (such as age at menarche and age at menopause) did not show material impact on the observed association, and thus were not included in the final model. Maximum-likelihood estimates of the parameters were obtained using SAS [37]. Tests for trends across smoking categories were conducted by using a likelihood ratio statistic in a logistic regression model.

Results

Table 1 presents the association between GSTM1, GSTT1 genotypes, and breast cancer risk. None of the GSTM1 genotypes was associated with breast cancer risk for either all women combined or among pre- or postmenopausal women. For GSTT1 a significantly increased breast cancer risk was observed among postmenopausal women with a GSTT1 null genotype (OR = 1.9, 95% CI 1.2–2.9). Cigarette smoking is not associated with the risk of female breast cancer in this study, among either pre- or postmenopausal women, or among current or past smokers (Table 2). There is also no clear risk pattern associated with various smoking characteristics (such as age when started smoking, amount, and duration of smoking) as shown in Table 2.

Table 3 presents the association between cigarette smoking and breast cancer risk by GSTM1 genotypes. There was no significantly increased risk associated with cigarette smoking for women with GSTM1A, GSTM1B, or GSTM1-null genotypes for all women combined. While there was a 40–50% increased risk for currently smoking women with GSTM1B and GSTM1-null genotypes, the risk was not statistically significant and did

Table 1. GSTM1 and GSTT1 genetic polymorphisms and breast cancer risk in Connecticut, 1998–2000

Genotypes	All			Premenopausal			Postmenopausal		
	Ca	Co	OR[a] (95% CI)	Ca	Co	OR[b] (95% CI)	Ca	Co	OR[b] (95% CI)
GSTM1									
A	106	102	1.2 (0.7–2.0)	30	36	1.0 (0.4–2.6)	76	66	1.4 (0.8–2.7)
B	41	44	1.0	13	15	1.0	28	29	1.0
A/B	5	14	0.4 (0.1–1.3)	1	6	0.2 (0.0–2.3)	4	8	0.6 (0.2–2.3)
Null	165	173	1.0 (0.6–1.7)	40	67	0.7 (0.3–1.7)	125	106	1.3 (0.7–2.4)
GSTT1									
Positive	223	259	1.0	60	90	1.0	163	169	1.0
Null	95	74	1.5 (1.0–2.2)	24	34	1.2 (0.6–2.3)	71	40	1.9 (1.2–2.9)

[a] Adjusted for age, BMI (<21, 21–24, >24), age of first full-term pregnancy (nulliparous, <20, 20–25, >25 years), lifetime months of breast feeding (never, 1–5, 6–11, ≥12 months) and family history of breast cancer, and by menopausal status.
[b] Without adjusting for menopausal status.

Table 2. Cigarette smoking and breast cancer risk by menopausal status in Connecticut, 1998–2000

Smoking	Premenopausal		Postmenopausal		All	
	Ca/Co	OR[a] (95% CI)	Ca/Co	OR[a] (95% CI)	Ca/Co	OR[a] (95% CI)
Never	45/61	1.0	97/93	1.0	142/154	1.0
Ever	42/67	0.8 (0.5–1.4)	154/124	1.2 (0.8–1.8)	196/191	1.1 (0.8–1.5)
Current	16/24	0.9 (0.4–2.0)	36/37	1.1 (0.6–2.0)	52/61	1.0 (0.6–1.6)
Past	26/43	0.8 (0.4–1.4)	118/87	1.3 (0.8–1.9)	144/130	1.1 (0.8–1.6)
Age started smoking (years)						
<18	23/38	0.8 (0.4–1.6)	77/61	1.2 (0.8–2.0)	100/99	1.1 (0.8–1.6)
18–24	15/27	0.7 (0.3–1.5)	63/54	1.2 (0.7–1.8)	78/81	1.0 (0.7–1.5)
>24	4/2	2.4 (0.4–14.9)	14/9	1.5 (0.6–3.7)	18/11	1.6 (0.7–3.7)
Amount of smoking per day						
<10	19/24	1.0 (0.5–2.1)	56/42	1.3 (0.8–2.1)	75/66	1.2 (0.8–1.8)
10–20	20/36	0.7 (0.4–1.5)	80/62	1.3 (0.8–2.0)	100/98	1.1 (0.8–1.6)
>20	3/7	0.6 (0.1–2.5)	18/20	0.9 (0.4–1.8)	21/27	0.8 (0.4–1.5)
Years of smoking						
<15	20/27	0.9 (0.4–1.9)	31/35	0.8 (0.5–1.5)	51/62	0.9 (0.6–1.4)
15–30	18/36	0.7 (0.3–1.4)	52/39	1.4 (0.8–2.3)	70/75	1.1 (0.7–1.6)
>30	4/4	1.2 (0.3–5.4)	71/50	1.4 (0.9–2.2)	75/54	1.3 (0.8–2.0)
Pack-years of smoking						
<5	17/27	0.8 (0.4–1.7)	36/29	1.1 (0.6–2.1)	53/56	1.0 (0.7–1.6)
5–20	21/28	1.0 (0.5–2.1)	60/47	1.3 (0.8–2.1)	81/75	1.2 (0.8–1.8)
>20	4/12	0.4 (0.1–1.4)	58/48	1.2 (0.7–1.9)	62/60	1.0 (0.6–1.5)

[a] Adjusted for age, BMI (<21, 21–24, ≥25), age of first full-term pregnancy (nulliparous, <20, 20–25, >25 years) and lifetime months of breast feeding (never, 1–5, 6–11, ≥12 months), and family history of breast cancer.

not show a clear risk pattern with age at which smoking began, duration of smoking, or lifetime pack-years. Stratification by menopausal status also showed no significantly increased risk by genotype among pre- or postmenopausal women (data not shown).

The relationship between cigarette smoking and breast cancer risk by GSTT1 genotypes is presented in Table 4. There was no increased risk of breast cancer associated with cigarette smoking for women with GSTT1-positive or GSTT1-null genotype for all subjects combined (Table 4). However, for postmenopausal women with GSTT1-null genotype there were indications of increased risk, although none of the results achieved statistical significance (Table 5). For example, "ever" smokers had a 90% insignificantly increased risk of breast cancer (OR = 1.9, 95% CI 0.8–4.4), and current smokers had a more than two-fold increased risk (OR = 2.3, 95% CI 0.6–8.9). Those who commenced smoking before age 18 showed a significantly increased risk (OR = 2.9, 95% CI 1.0–8.8). Risk was also in-

Table 3. GSTM1 genotypes, cigarette smoking, and breast cancer risk in Connecticut, 1998–2000

Smoking	GSTM1-A		GSTM1-B		GSTM1-null	
	Ca/Co	OR[a] (95% CI)	Ca/Co	OR[a] (95% CI)	Ca/Co	OR[a] (95% CI)
Never	51/46	1.0	15/17	1.0	62/76	1.0
Ever	55/56	1.0 (0.5–1.7)	26/27	1.1 (0.4–2.8)	103/97	1.2 (0.8–1.9)
Current	11/22	0.5 (0.2–1.3)	7/5	1.4 (0.3–6.2)	33/29	1.5 (0.8–2.9)
Past	44/34	1.2 (0.7–2.3)	19/22	1.0 (0.4–2.7)	70/68	1.1 (0.7–1.8)
Age started smoking (years)						
<18	24/33	0.8 (0.4–1.6)	15/15	1.1 (0.4–3.4)	57/45	1.5 (0.9–2.6)
18–24	24/19	1.1 (0.5–2.3)	7/10	0.8 (0.2–3.0)	41/47	1.0 (0.5–1.7)
>24	7/4	1.7 (0.4–6.5)	4/2	1.9 (0.2–14.6)	5/5	1.0 (0.3–3.8)
Amount of smoking per day						
<10	18/20	0.9 (0.4–2.1)	10/8	1.6 (0.5–5.4)	41/32	1.5 (0.8–2.6)
10–20	30/26	1.1 (0.6–2.2)	13/17	0.7 (0.2–2.3)	52/51	1.2 (0.7–2.0)
>20	7/10	0.7 (0.2–2.0)	3/2	2.1 (0.3–17.5)	10/14	0.8 (0.3–2.0)
Years of smoking						
<15	15/16	0.9 (0.4–2.1)	9/15	0.7 (0.2–2.1)	25/29	1.1 (0.5–2.0)
15–30	25/22	1.3 (0.6–2.7)	8/6	1.8 (0.5–7.0)	34/41	1.1 (0.6–1.9)
>30	15/18	0.7 (0.3–1.7)	9/6	1.6 (0.4–7.1)	44/27	1.6 (0.8–2.9)
Pack-years of smoking						
<5	12/17	0.6 (0.3–1.6)	7/9	1.0 (0.3–3.5)	31/26	1.4 (0.8–2.8)
5–20	27/22	1.5 (0.7–3.1)	10/10	1.1 (0.3–4.0)	40/39	1.2 (0.7–2.1)
>20	16/17	0.8 (0.3–1.8)	9/8	1.1 (0.3–4.3)	32/32	1.0 (0.6–2.0)

[a] Adjusted for age, BMI (<21, 21–24, >24), age of first full-term pregnancy (nulliparous, <20, 20–25, >25 years), lifetime months of breast feeding (never, 1–5, 6–11, ≥12 months), family history of breast cancer, and menopausal status.

Table 4. GSTT1 genotypes, cigarette smoking, and breast cancer risk in Connecticut, 1998–2000

Smoking	GSTT1-positive		GSTT1-null	
	Ca/Co	OR[a] (95% CI)	Ca/Co	OR[a] (95% CI)
Never	90/111	1.0	41/35	1.0
Ever	132/149	1.0 (0.7–1.5)	54/39	1.2 (0.6–2.3)
Current	37/44	1.1 (0.6–1.9)	14/14	1.1 (0.4–2.7)
Past	95/105	1.0 (0.7–1.5)	40/25	1.3 (0.6–2.6)
Age started smoking (years)				
<18	66/79	1.0 (0.7–1.6)	32/17	1.7 (0.8–3.7)
18–24	53/60	1.0 (0.6–1.6)	19/20	0.8 (0.4–1.9)
>24	13/10	1.3 (0.5–3.3)	3/2	0.8 (0.1–6.3)
Amount of smoking per day				
<10	48/49	1.2 (0.7–1.9)	22/16	1.2 (0.5–3.0)
10–20	69/77	1.1 (0.7–1.7)	27/20	1.2 (0.5–2.6)
>20	15/23	0.8 (0.4–1.6)	5/3	1.2 (0.2–6.0)
Years of smoking				
<15	37/48	0.9 (0.5–1.5)	12/15	0.7 (0.3–1.8)
15–30	43/59	0.9 (0.6–1.5)	25/15	1.6 (0.7–3.9)
>30	52/42	1.4 (0.8–2.3)	17/9	1.3 (0.5–3.6)
Pack-years of smoking				
<5	35/40	1.0 (0.6–1.8)	16/16	1.0 (0.4–2.5)
5–20	58/62	1.2 (0.7–1.9)	19/13	1.2 (0.5–3.0)
>20	39/47	0.9 (0.5–1.5)	19/10	1.4 (0.5–3.7)

[a] Adjusted for age, BMI (<21, 21–24, >24), age of first full-term pregnancy (nulliparous, <20, 20–25, >25 years), lifetime months of breast feeding (never, 1–5, 6–11, ≥12 months), family history of breast cancer, and menopausal status.

Table 5. Breast cancer risk for postmenopausal women by GSTT1 genotype and cigarette smoking

Smoking	GSTT1-positive		GSTT1-null	
	Ca/Co	OR[a] (95% CI)	Ca/Co	OR[a] (95% CI)
Never	61/67	1.0	28/21	1.0
Ever	101/103	1.1 (0.7–1.7)	43/19	1.9 (0.8–4.4)
Current	25/29	1.1 (0.6–2.1)	10/6	2.3 (0.6–8.9)
Past	76/74	1.1 (0.7–1.8)	33/13	1.7 (0.7–4.4)
Age started smoking (years)				
<18	50/52	1.1 (0.6–1.9)	25/7	2.9 (1.0–8.8)
18–24	42/43	1.1 (0.6–1.9)	15/10	1.2 (0.4–3.6)
>24	9/8	1.1 (0.4–3.1)	3/2	1.3 (0.1–11.4)
Amount of smoking per day				
<10	34/36	1.0 (0.6–1.9)	17/5	2.9 (0.8–10.4)
10–20	55/50	1.2 (0.7–2.1)	21/12	1.4 (0.5–3.9)
>20	12/17	0.8 (0.3–1.8)	5/2	2.0 (0.3–13.3)
Years of smoking				
<15	21/31	0.7 (0.4–1.4)	8/5	1.1 (0.3–4.5)
15–30	32/32	1.2 (0.6–2.2)	18/7	2.0 (0.6–6.7)
>30	48/40	1.3 (0.8–2.3)	17/7	2.3 (0.7–7.4)
Pack-years of smoking				
<5	23/26	0.9 (0.5–1.8)	11/3	2.3 (0.5–10.5)
5–20	42/39	1.3 (0.7–2.2)	14/8	1.5 (0.5–4.9)
>20	36/38	1.0 (0.6–1.9)	18/8	2.0 (0.7–5.9)

[a] Adjusted for age, BMI (<21, 21–24, ≥25), age of first full-term pregnancy (nulliparous, <20, 20–25, >25 years), and lifetime months of breast feeding (never, 1–5, 6–11, ≥12 months), and family history of breast cancer.

creased among those who smoked for more than 30 years (OR = 2.3, 95% CI 0.7–7.4), and who had a lifetime of more than 20 pack-years of smoking (OR = 2.0, 95% CI 0.7–5.9), although neither achieved statistical significance. We were unable to evaluate the smoking effects among premenopausal women based on GSTT1 genotypes due to the small sample size.

We found no significantly increased risk of breast cancer associated with cigarette smoking for any combinations of the GSTM1 and GSTT1 genotypes (Table 6). Smokers who lacked both GSTT1 and GSTM1 genotypes had odds ratios of 1.7 (95% CI 0.6–5.1) for all women combined, and 3.0 for postmenopausal women (95% CI 0.7–12.5). Smokers with GSTT1-null and GSTM1B genotypes had odds ratios of 2.2 (95% CI 0.1–53.0) for all women combined, and 3.5 (95% CI 0.4–31.9) for postmenopausal women, based on seven cases and three controls. No increased risk was observed for subjects with GSTT1-positive genotype and the three GSTM1 genotype combinations.

Table 6. GSTM1, GSTT1, cigarette smoking, and breast cancer risk in Connecticut, 1998–2000

GST genotypes	Never		Ever	
	Ca/Co	OR	Ca/Co	OR[a] (95% CI)
Total				
GSTT1-null				
GSTM1-null	17/18	1.0	29/22	1.7 (0.6–5.1)
GSTM1-A	17/12	1.0	17/10	1.1 (0.3–3.7)
GSTM1-B	5/4	1.0	8/5	2.2 (0.1–52.5)
GSTT1-positive				
GSTM1-null	45/58	1.0	74/75	1.2 (0.7–2.1)
GSTM1-A	34/34	1.0	38/46	0.9 (0.5–1.8)
GSTM1-B	10/13	1.0	18/22	0.9 (0.3–2.8)
Postmenopausal[b]				
GSTT1-null				
GSTM1-null	13/9	1.0	25/8	3.0 (0.7–12.5)
GSTM1-A	10/7	1.0	11/7	0.7 (0.1–4.8)
GSTM1-B	3/4	1.0	7/3	3.5 (0.4–31.9)
GSTT1-positive				
GSTM1-null	29/36	1.0	58/53	1.3 (0.7–2.6)
GSTM1-A	27/22	1.0	28/30	0.9 (0.4–2.0)
GSTM1-B	5/7	1.0	13/15	1.5 (0.3–8.1)

[a] Adjusted for age, BMI (<21, 21–24, ≥25), age at first full-term pregnancy (nulliparous, <20, 20–25, >25 years), lifetime duration of lactation (never, 1–5, 6–11, ≥12 months), family breast cancer history, and menopausal status.

[b] Without adjusting for menopausal status.

Discussion

Our study did not find an increased risk of breast cancer associated with any of the three GSTM1 genotypes (GSTM1A or GSTM1B or GSTM1-null), and these genotypes did not show a major impact on the relation-

ship between cigarette smoking and breast cancer risk. GSTT1-positive genotype also showed no increased risk of breast cancer either by itself or in combination with cigarette smoking. There was, however, some suggestion that the GSTT1-null genotype was associated with an increased risk of breast cancer, particularly for postmenopausal women, and for women who started smoking at younger ages.

It has been postulated that cigarette smoking may be either anticarcinogenic or procarcinogenic with respect to breast cancer risk. MacMahon *et al.* [38] initially hypothesized that cigarette smoking may reduce breast cancer risk due to its possible antiestrogenic effects in women. This was based on studies that found that female smokers had an earlier age at menopause, a decreased risk of endometrial cancer, increased incidence of osteoporosis, and lower levels of endogenous estrogens as reviewed by Calle *et al.* [31]. However, this evidence is indirect, and more direct studies of hormonal status in smokers and nonsmokers failed to demonstrate a consistent association [1, 3]. In our study we also found no evidence of an inverse association between cigarette smoking and breast cancer risk for any of the genotypes studied.

By contrast, Hiatt and Fireman [39] have suggested that cigarette smoking may have a direct carcinogenic effect on the breast, which could outweigh the possible antiestrogenic effect of smoking. This possibility is supported by the demonstration of DNA adducts of PAHs in human breast cells [6, 7], the presence of mutagens in the nipple aspirates of nonlactating women, and the finding of nicotine in the breast fluid in considerably greater concentrations than in the plasma of smokers, as reviewed by Palmer and Rosenberg [3]. Experimental studies have also shown that PAHs cause mammary tumors in rodents [4, 5].

It is interesting to note that in the present study we observed that postmenopausal women who had a GSTT1-null genotype had a significantly increased risk of breast cancer if they began smoking in their early teenage years. This observation is consistent with those of the earlier studies indicating that cigarette smoking commencing at younger ages was associated with an increased risk of breast cancer [1, 10, 40].

Our study found an association between breast cancer risk and early smoking only in the GSTT1-null subjects, and not the GSTM1-null genotype subjects. These results provide some support to those of Rebbeck [13], who reported accelerated age of first breast cancer diagnosis among GSTT1-null genotype, and found no association with GSTM1 genotype. A potential higher risk of breast cancer associated with GSTT1-null genotype is also supported in a recent study by Norppa *et al.* [41], who found that lack of the GSTT1 gene determines individual sensitivity to the genotoxic and cytotoxic effects of diepoxybutane (DEB), while the absence of GSTM1 does not. Consequently, they suggest that glutathione conjugation mediated by GSTT1, not GSTM1, is likely to be the major detoxification pathway for DEB in cultured human lymphocytes. Therefore, whether lack of GSTT1 activity also determines the risk of breast cancer from tobacco smoking warrants further investigation.

While a combined analysis of earlier studies by Dunning *et al.* [42] showed that the GSTM1 gene deletion was significantly associated with postmenopausal breast cancer (null homozygote OR = 1.3, $p = 0.04$), a majority of the more recent studies with larger sample sizes, however, found no increased risk of breast cancer associated with GSTM1 polymorphisms [22, 25, 43, 44–47]. In one study by Park *et al.* [21], breast cancer risk was found to be increased for GSTM1-null genotype only in premenopausal women, but not in postmenopausal women. Conversely, another study by Mitrunen *et al.* [23] found a significant association only between GSTM1-null genotype and postmenopausal breast cancer.

A lack of association between breast cancer risk and GSTM1 genotype seems surprising given the GSTM1 gene's role in metabolizing carcinogenic compounds, such as PAHs, and their consistent association with lung and bladder cancer from tobacco smoking. One potential explanation suggested by Rebbeck [13] is that the GSTs may exert tissue- or site-specific effects or may predispose to specific cancer subtypes (such as postmenopausal but not premenopausal breast cancer, squamous cell carcinoma but not adenocarcinoma of the lung). In support of the hypothesis, GSTM1 is expressed in breast tissue at low levels, but expressed at relatively high levels in lung and bladder tissue [48], and GSTM1-null genotype has also been most consistently associated with higher risk of lung and bladder cancers [13].

The association between GSTT1 polymorphism and breast cancer risk is also inconsistent from earlier epidemiological studies. A few studies found no significantly increased risk of breast cancer associated with GSTT1 polymorphism [25, 42, 43, 45–47]. The study by Garcia-Closas *et al.* [22] suggested a decreased risk of breast cancer associated with the GSTT1-null genotype among premenopausal women. There are, however, four recent studies suggesting an increased risk of breast cancer associated with GSTT1-null genotype either independently or in combination with other GST genotypes [20, 21, 23, 44]. For example, Park *et al.* [21] reported an OR of 1.6 (95% CI 1.0–2.5) for GSTT1-null genotype for breast cancer risk, with an increased risk (OR = 2.2, 95% CI 1.1–4.5) for women with a

concurrent lack of both GSTM1 and GSTT1 genes. A borderline significant increase in the risk of breast cancer was also reported [23] for premenopausal women with the combination of GSTM1-null, GSTP1 Ile/Ile, and GSTT1-null genotypes (OR = 4.0, 95% CI 1.0–15.8). The study by Gudmundsdottir *et al*. [44], found no association with the GSTM1-null genotype; however, it did find that GSTT1 genes could play a role in carcinogenesis in the breast, possibly through an increased frequency of mutations in tumor-suppressor genes such as p53.

Chance may explain the inconsistent results linking GST genotypes, cigarette smoking, and breast cancer risk. The sample sizes in most of the earlier studies – that investigated the association between GSTs, cigarette smoking and breast cancer risk – have been relatively small. Although our study had a sample size of 338 cases and 345 controls, the number of subjects is still relatively small, especially after stratification by GST genotype and menopausal status. This limits our power to demonstrate an interaction between the effects of these genotypes and cigarette smoking. Studies with larger sample sizes from different populations are clearly needed to address the issue.

In summary, the results from this study suggest that women with GSTM1-null genotype do not have an increased risk of breast cancer resulting from cigarette smoking. The data, however, suggest that women with a GSTT1-null genotype may have an increased breast cancer risk, especially for postmenopausal women who started smoking at younger ages. An increased risk of breast cancer associated with cigarette smoking is biologically plausible. Clearly, further study of the interaction between GSTT1 genotype and cigarette smoking in the development of breast cancer is warranted.

Acknowledgement

This study is supported by grants CA-62986 and CA-81810 from the National Cancer Institute and the National Institute of Environmental Health Science.

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